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TECHNICAL MANUSCRIPT 559

CANDIDIASIS IN SIMIANS

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SEPTEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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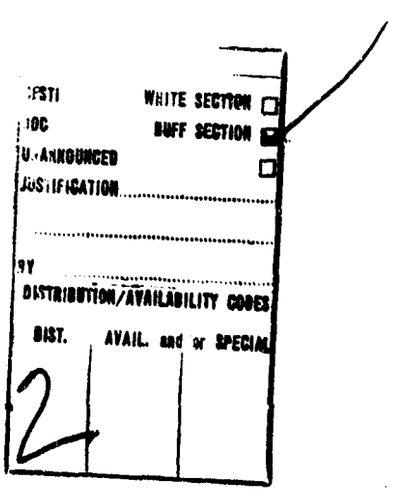

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CANDIDIASIS IN SIMIANS

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MEDICAL SCIENCES LABORATORIES
and
Animal Farm Division
AEROBIOLOGY & EVALUATION LABORATORIES

Project 1B562602AD01

September 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Candidiasis was diagnosed in six monkeys over a 10-month period. Most cases had been on antibiotic therapy for enterocolitis. Fungal invasion was seen in epithelium of the tongue, oral cavity, esophagus, and colon, and in hard keratin of the nails. Gross lesions of the anterior alimentary tract were either white patches or ulcers of the mucosa. Lesions of the colon consisted of a thick pseudomembrane that contained numerous Candida. The nails exhibited typical Candida onychomycosis. C. albicans was isolated from the two cases that were cultured. Tissue invasion by Candida blastospores and hyphae was histologically demonstrated in all cases.

I. INTRODUCTION*

Fungi of the genus Candida are common saprophytes of the skin, alimentary tract, and reproductive tract of monkeys. Candida spp. were isolated from 8 to 100% of healthy monkeys cultured in 10 surveys[1-10] and C. albicans was the species most frequently recovered. The common sites of isolation were the oral cavity, rectum, vagina, and skin.

As a saprophyte, Candida is found in keratin of the stratum corneum of epithelium and in digestive tract contents. Occasionally it has been isolated from soil or vegetable sources and is considered an obligate saprophyte because it does not propagate outside the body.[11] Numerous debilitating conditions enable this facultative pathogen to produce serious and even fatal disease in primates.

We have found reports in the literature of six cases of candidiasis in subhuman primates. Candida albicans has been isolated from tongue lesions by Thiry[12] in a vervet monkey (Ceracopithecus patas) experimentally infected with Trypanosoma cruzi and by Fiennes[13] in a wooly monkey (Lagothrix sp.) and a gorilla (Gorilla gorilla beringei). Fiennes also recovered C. albicans from the tongue, mouth, lungs, liver, and intestine of a capuchin (Cebus sp.) that died of systemic candidiasis. Saëz[14] cultured C. albicans from a barbary ape (Macaca sylvana) with a lung condition. Kerber, Reese, and Van Natta[15] isolated C. albicans from the penis and hand of a rhesus monkey (Macaca mulatta) with balanitis, paronychia, and onychia that responded to antifungal therapy.

We feel that the frequency of clinical candidiasis in simians is greater than the literature indicates. The present paper describes the clinical signs, gross and microscopic lesions, and diagnostic mycology of spontaneous candidiasis in six monkeys.

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II. MATERIALS AND METHODS

Cases 1 to 5 are rhesus monkeys (M. mulatta) that became ill during the 3-month conditioning period at the Fort Detrick Animal Farm. Case 6 is a capuchin (Cebus albifrons) that died at the quarantine facility of a local pet shop. The Fort Detrick monkeys were maintained on a preventive disease regimen consisting of tuberculin testing and intramuscular injection of benzathine penicillin G upon arrival, prophylactic levels of oxytetracycline* in the feed** for 2 weeks after arrival, and administration of thiabendazole 6 and 9 weeks following arrival. Standard diarrhea therapy consisted of intramuscular vitamin B complex and antibiotics combined with an oral preparation of furazolidone, kaolin pectate, electrolytes,*** and 10% dextrose administered via stomach tube. Chloramphenicol, kanamycin, and ampicillin were the commonly administered antibiotics. Duration of treatment varied from 2 to 6 consecutive days. Bacterial culture, antibiotic sensitivity tests, egg flotation, and direct smears for parasites were conducted on fecal samples of nonresponsive cases.

Monkeys with ulcers or elevated white plaques of the tongue or oral cavity were considered candidiasis suspects. Two cases were clinically diagnosed by direct smears of lesions in 10% NaOH stained with methylene blue and cultured on Sabouraud's agar. C. albicans was identified by sugar fermentation tests[16] in case 5 and by production of chlamydospores on chlamydospore agar**** in case 6.

To determine virulence, rabbits were injected with the isolate from case 6. Serial tenfold dilutions were made from a saline suspension of a 48-hour culture grown on Sabouraud's agar. Three-pound rabbits were injected intracardially with 0.5 ml of the 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-5} dilutions. To estimate the number of organisms inoculated, 0.1 ml of each dilution was streaked on plates of Sabouraud's agar.

All monkeys were necropsied and tissue sections were routinely stained with hematoxylin and eosin; Gomori methenamine silver or Gridley's fungus stains were used to vividly color fungi.[17]

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* Terramix A-B-D-25®, Chas. Pfizer & Co., Inc., New York, New York.
** Purina Monkey Chow, Ralston Purina Co., St. Louis, Mo.
*** Vetrad-O®, Don Hall Laboratories, Norwich, New York.
**** Chlamydospore agar, Baltimore Biological Laboratories, Baltimore, Md.

III. RESULTS

Candidiasis was diagnosed in the six monkeys over a period of 10 months. Predisposing conditions were present in five cases. Cases 1 to 4 had dysentery and had undergone several days' antibictic and symptomatic therapy prior to death. Cases 1 and 3 had been successfully treated for dysentery several weeks before the final illness. Case 6 had a systemic viral infection microscopically characterized by intranuclear and cytoplasmic, eosinophilic inclusion bodies in reticuloendothelial cells of the lymphoid system. Case 5 was dyspneic and C. albicans was isolated from pharyngeal ulcers. This monkey was sacrificed to determine the relationship of the fungus to the lesions.

Clinical signs of candidiasis varied with the location of lesions. Fungal invasion was seen in epithelium of the tongue, oral cavity, esophagus, and colon, and in hard keratin of the nails. Ulcers or white, slightly elevated, rough-surfaced plaques of the tongue or oral cavity were seen on physical examination in cases 2, 5, and 6. Plaques varied in size from 0.3 to 1 cm in diameter in case 5 to a white covering of the entire dorsum of the tongue in case 6. The lesions sometimes were difficult to see grossly, as in case 2, which had mild superficial sloughing of small areas of the posterior dorsum of the tongue. Open-mouth breathing occurred in case 5, which had ulcers 0.5 to 1 cm in diameter on a swollen soft palate. Case 2 had a severe ulcerative esophagitis that caused dysphagia. Upon necropsy each of the above cases had oral lesions and white longitudinal streaks 1 to 2 mm wide in the esophageal mucosa. Microscopically, the white plaques consisted of Candida and bacteria multiplying in a greatly thickened stratum corneum (Fig. 1). Oval blastospores were located near the surface and thin septate hyphae penetrated the stratum spinosum (Fig. 2). Neutrophils were located in pockets of the stratum corneum and in clefts between it and the stratum spinosum. There were hyperplastic rete pegs and a mild infiltration of mononuclear cells in the dermal papillae in involved areas (Fig. 3). Cases 1 and 3 had microscopic candidiasis of the tongue. Invasion of the lamina propria by hyphae was seen in the ulcerated esophagus of case 2 (Fig. 4-6). Cellular debris and myriad blastospores partially occluded its lumen.

The colon was involved in cases 2 and 4. The lumen of the terminal descending colon and rectum of case 4 was partially occluded by a thick layer of firm pink material that adhered to the mucosa. Microscopically this material was a pseudomembrane consisting of degenerating epithelial cells, leukocytes, bacteria, and large numbers of Candida blastospores and hyphae lodged in an acidophilic proteinaceous matrix (Fig. 7 and 8). The underlying mucosa was acutely inflamed. Massive Candida colonization was seen in the lumen of the inflamed colon of case 2.

FIGURE 1. Dorsolateral Portion of Tongue with Greatly Thickened Stratum Corneum. Case 6; H & E, 42X.

FIGURE 2. Blastospores Near the Surface and Hyphae Penetrating Through the Thickened Stratum Corneum into the Stratum Spinosum of the Tongue. Case 6; GMS, 130X.

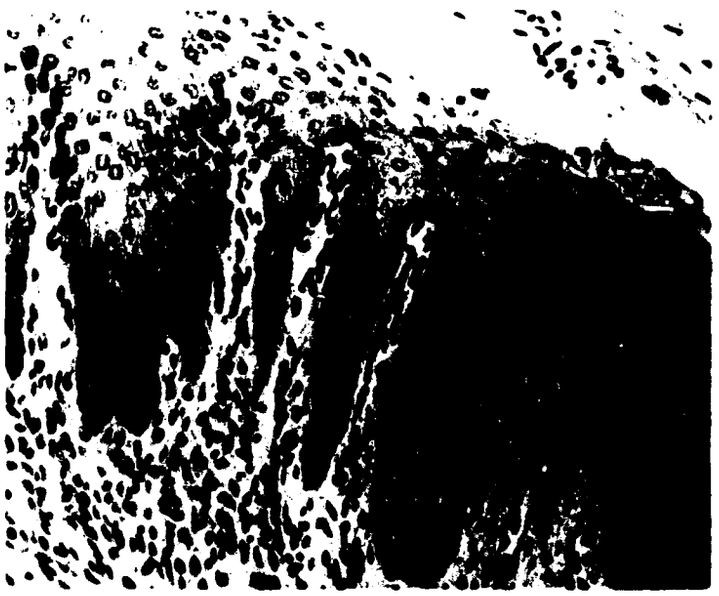

FIGURE 3. Hyperplastic Rete Pegs and Infiltration of Mononuclear Cells in Dermal Papillae of the Epithelium of the Tongue. Case 6; H & E, 420X.

FIGURE 4. Ulcerated Esophagus with Destruction of Most of the Mucosal Epithelium. The lumen is partially occluded by cellular debris and Candida. Case 2; H & E, 42X.

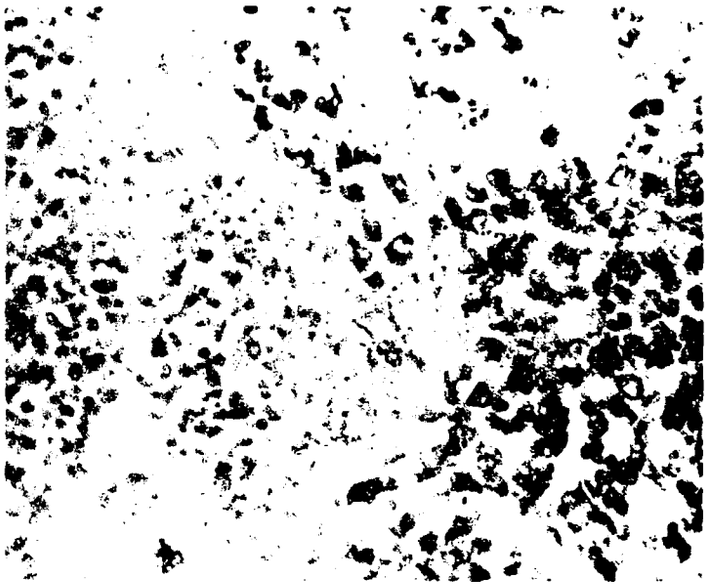

FIGURE 5. Numerous Candida Blastospores Adjacent to Degenerating Inflammatory Cells within the Esophagus. Case 2; H & E, 420X.

FIGURE 6. Hyphae of Candida Penetrating the Lamina Propria of the Ulcerated Esophagus. Case 2; GMS, 420X.

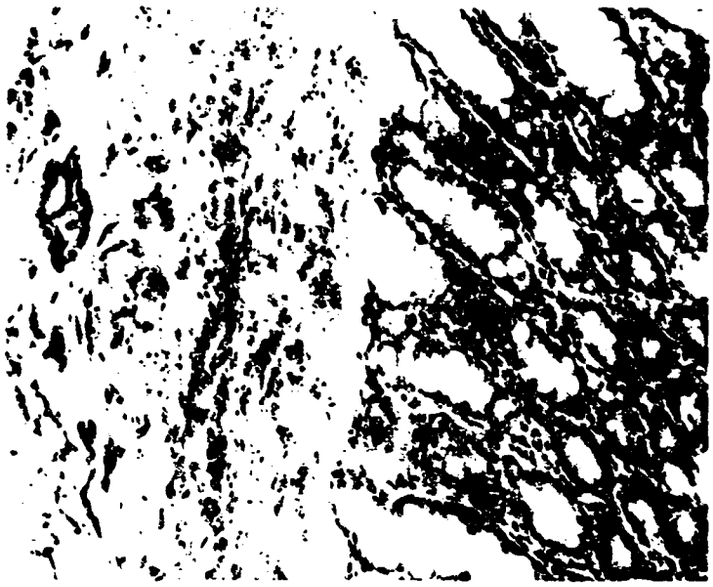

FIGURE 7. Pseudomembrane Adherent to Inflamed Colon. The proteinaceous material within dilated crypts of Lieberkühn is identical to the ground substance of the pseudomembrane. Case 4; H & E, 130X.

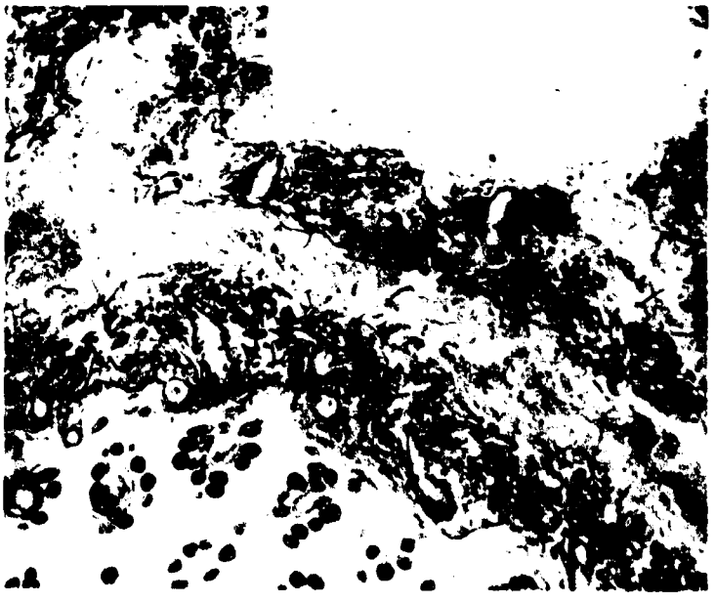

FIGURE 8. Blastospores and Hyphae in Pseudomembrane of the Colon. Case 4; GMS, 130X.

Candida onychomycosis occurred in case 1. The fingernails and toenails were eroded, shortened, and deformed. This monkey also had circular shallow ulcers on the fingertips and toes and a focally inflamed prepuce. Microscopic examination of the deformed nails revealed a disruption of the layers of keratin by Candida blastospores and hyphae (Fig. 9 and 10). The digital ulcers and posthitis were primarily bacterial inflammations with secondary surface growth of Candida.

Candidiasis was clinically diagnosed in cases 5 and 6. Filamentous hyphae and budding yeasts were seen in direct smears of lesions stained with methylene blue. Culture on Sabouraud's agar at 25 C produced smooth, raised, white colonies with a yeast-like odor within 36 hours. The isolates were identified as C. albicans in case 5 by the production of acid and gas in selected sugars[18] and in case 6 by the production of chlamydospores (Fig. 11). Approximately 375,000 organisms of this isolate* inoculated intracardially into a rabbit produced death in 5 days, with disseminated abscesses 1 to 2 mm in diameter. The organism was recovered by culture from the abscesses and microscopically demonstrated in tissue section.

IV. DISCUSSION

These cases illustrate several general features of Candida infections. This opportunistic fungus is capable of living as a saprophyte or pathogen. Candidiasis, with serious, and sometimes fatal, manifestations has occurred with many conditions that result in an impairment of the body's defense mechanisms. Neoplastic and infectious diseases, immunologic defects, and conditions requiring antibiotic, corticosteroid, antimetabolite, or radiation therapy are included in this category.[18] The examples presented are cases of infectious disease treated with antibiotics, with subsequent invasion by Candida. Recently there have been excellent discussions on the interrelationships of Candida, bacteria, and antibiotics.[18,19]

The role of Candida as a secondary invader should not diminish our concept of its significance. We feel that its clinical effects can be more severe than those of the suspected underlying process. The ulcerative esophagitis of case 2 contributed to its fatal outcome. Esophageal candidiasis should be considered in dysphagic monkeys, especially if oral candidiasis lesions are present. Candida produces a potent endotoxin-like substance that has produced fatal shock when injected intravenously in mice.[20,21] The extent of local damage or adverse systemic effects by this toxin in instances of massive intestinal colonization as seen in cases 2 and 5 is not known.

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* Identification of culture from case 6 was confirmed by National Animal Disease Laboratory, Ames, Iowa.

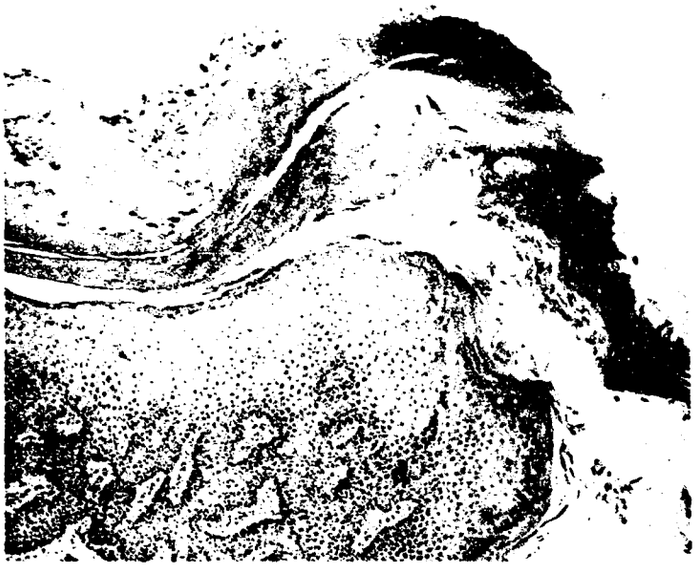

FIGURE 9. Shortened, Deformed Fingernail. Case 1; H & E, 42X.

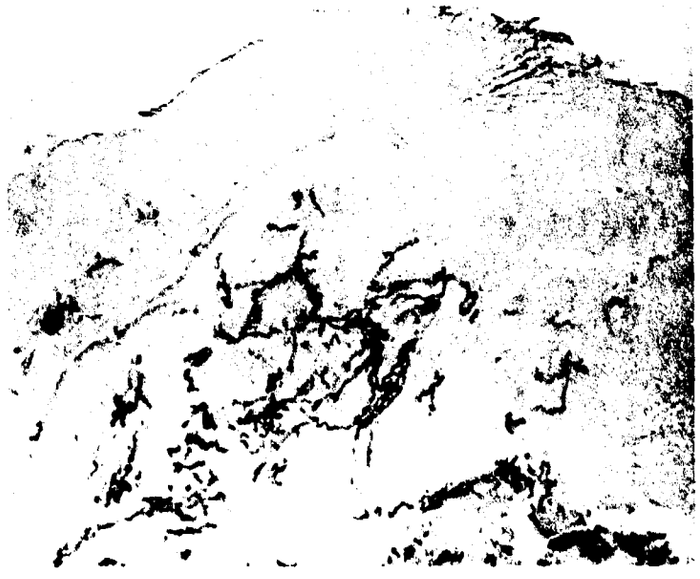

FIGURE 10. Blastospores and Hyphae of Candida within the Keratin of the Nail. Case 1, Gridley's fungus stain, 420X.

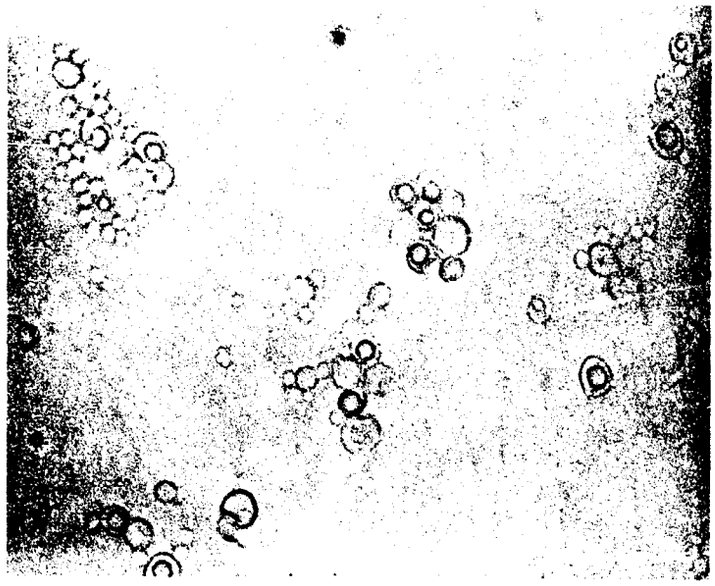

FIGURE 11. Thick-Walled Chlamydospores Characteristic of C. albicans Grown on Chlamydospore Agar. Case 6; slide culture, 130X.

Differential diagnosis of Candida tongue lesions includes ulcers associated with herpes virus infections[22] and monkey pox.[23] The common saprophytic occurrence of Candida throughout the body limits the diagnostic value of cultures. Cultures are considered pathognomonic only when they originate from a tissue or fluid, such as blood, that does not normally harbor Candida.[24] It is thought that in vivo the yeast form of Candida is saprophytic and the hyphal form is invasive.[25] The demonstration of hyphae in fecal smears indicates intestinal candidiasis.[26] In biopsy or necropsy tissue sections, blastospores and hyphae can be identified satisfactorily as members of the genus Candida.[27] Species identification requires culture and differential tests.

Superficial candidiasis can be treated successfully if antifungal therapy is combined with correction of predisposing factors. Skin and vaginal infections respond well to topical nystatin or chlordantoin.[28] Nail infections are generally refractory to treatment, although nystatin is sometimes effective.[15,16] Oral nystatin is very effective for digestive tract candidiasis.[26] The drug of choice for systemic candidiasis, which has a poor prognosis, is intravenous amphotericin B.[28]

A correlation between positive Candida and tuberculin skin tests in guinea pigs harboring Candida was found by Vogel et al.[29] Matsufuji, Fukunda, and Nakamura[30] demonstrated in vivo that a common antigen is shared by C. albicans and Mycobacterium tuberculosis. This raises the possibility that C. albicans may be the cause of false positive tuberculin reactions in monkeys.

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Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701	Unclassified 2b. GROUP

3. REPORT TITLE

CANDIDIASIS IN SIMIANS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

Steven E. Wikse Robert M. Kovatch
James G. Fox

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1969	21	30

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B562602AD01	Technical Manuscript 559
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) CMs 6592
d.	

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13. ABSTRACT

Candidiasis was diagnosed in six monkeys over a 10-month period. Most cases had been on antibiotic therapy for enterocolitis. Fungal invasion was seen in epithelium of the tongue, oral cavity, esophagus, and colon, and in hard keratin of the nails. Gross lesions of the anterior alimentary tract were either white patches or ulcers of the mucosa. Lesions of the colon consisted of a thick pseudomembrane that contained numerous Candida. The nails exhibited typical Candida onychomycosis. C. albicans was isolated from the two cases that were cultured. Tissue invasion by Candida blastospores and hyphae was histologically demonstrated in all cases.

14. Key Words

Candidiasis
Monkeys
Laboratory animals
Fungus
Candida albicans
Histopathology

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